

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
1 July 2004 (01.07.2004)

PCT

(10) International Publication Number
WO 2004/054549 A1

(51) International Patent Classification⁷: **A61K 9/51**,
9/06, 47/14, 31/585

(21) International Application Number:
PCT/GB2002/005680

(22) International Filing Date:
13 December 2002 (13.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant (for all designated States except US):
JAGOTEC AG [CH/CH]; Eptingerstrasse 51, CH-4132
MuttENZ (CH).

(71) Applicant (for LC only): BASSIL, Nicholas, Charles
[GB/GB]; Kilburn & Strode, 20 Red Lion Street, London
WC1R 4PJ (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only):~ VERGNAULT,
Guy [FR/FR]; 9, rue des Merles, F-68680 Kembs Loechle
(FR).~GRENIER, Pascal [FR/FR]; Résidence la Clef
des Champs, 5, rue des Vignes, F-68510 Kappelen
(FR).~NHAMIAS, Alain [FR/FR]; 10, rue des Bleuets,
F-68870 Bartenheim (FR).~SCHERER, Dieter [DE/CH];
SkyePharma AG, Eptingerstrasse 51, CH-4132 MuttENZ
(CH).~BECK, Petra [DE/CH]; SkyePharma AG, Eptinger-
strasse 51, CH-4132 MuttENZ (CH).~CANCADE, Patricia
[FR/FR]; Residence les Jardins d'Eden, 6, rue de l'Ancien
Golf, F-68300 Saint Louis (FR).

(74) Agents: BASSIL, Nicholas, Charles et al.; Kilburn &
Strode, 20 Red Lion Street, London WC1R 4PJ (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i)) for the fol-
lowing designations AE, AG, AL, AM, AT, AU, AZ, BA, BB,
BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK,
DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,
OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM,
TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW,
ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ,
DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii)) for the following designations AE,
AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ,
VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS,
MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB,
GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
SN, TD, TG)
- of inventorship (Rule 4.17(iv)) for US only

Published:

- with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: A TOPICAL NANOPARTICULATE SPIRONOLACTONE FORMULATION

(57) Abstract: The invention relates to a topical nanoparticulate spironolactone formulation comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of from about 300nm to about 900nm. The nanoparticles are incorporated into a crystalline network system comprising a dispersion of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards the spironolactone nanoparticles.

A TOPICAL NANOPARTICULATE SPIRONOLACTONE FORMULATION.

5 The present invention relates to the use of spironolactone in the form of nanoparticles in the topical treatment of a condition responding to anti-androgens. Such conditions include acne, hirsutism, androgenic alopecia or rosacea.

10 Spironolactone is known as an aldosterone inhibitor having utility as a potassium sparing diuretic. It is commercially available as e.g. aldactone and may be employed e.g. in the treatment of congestive heart failure. Spironolactone has extremely low solubility in water, viz: 2.8mg/100ml. This low solubility can adversely affect absorption of the drug substance *in vivo*, leading to poor bioavailability. Consequently higher amounts of the drug substance are required to achieve the desired blood levels. The poor solubility
15 of spironolactone also restricts the options available for formulating the drug substance.

Other pharmaceutical applications make use of the anti-androgenic effects of Spironolactone for the treatment of a variety of skin disorders such as acne, hirsutism, androgenic alopecia and rosacea. Topical administration for these
20 disorders would be the preferred route due to the greatly reduced systemic side effects. However, again it is the poor solubility of the drug, which limits the development of efficacious and aesthetically acceptable topical formulations.

25 Following oral administration, the absorption of drugs from the intestine is mainly dependent on their solubility in the intestinal fluids and their intestinal permeability. Poorly soluble drugs generally have low dissolution rates and

exhibit only a small concentration gradient across the intestinal mucosa, which can result in low and unreliable levels of absorption. Drug substances which have low solubility also suffer from disadvantages in respect of other routes of administration, for example, topically.

5

Significant efforts have been directed to producing drug substances in the form of microparticles and nanoparticles. However, preparation of such small particles is not a trivial matter and can give rise to further difficulties both in relation to technical aspects of the process and in obtaining a satisfactory product. Thus for example there can be difficulties, especially on a manufacturing scale in obtaining a consistent and narrow particle size range. Furthermore, it is necessary to obtain stable products, e.g. nanosuspensions, but microparticles and nanoparticles have a tendency to aggregate and flocculate, which has adverse consequences for the stability of the product. A number of different approaches have been investigated for the preparation of microparticles and nanoparticles.

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US Patent 5,091,188 describes a method for preparing injectable solutions of water-insoluble drugs, which comprises reducing the crystalline drug substance to dimensions in the range 50nm to 10µm, by sonication or other processes inducing high shear, in the presence of a phospholipid or other membrane-forming amphipathic lipid, whereby the drug microcrystals become coated with said lipid.

20

US Patent No 5,145,684 describes particles of crystalline drug substance having a non-cross linked surface modifier adsorbed on the surface and an effective average particle size of less than about 400nm. These particles are said to be

25

prepared by milling in the presence of grinding media, using for example a ball mill, an attrition mill, a vibratory mill or a media mill.

5 International Patent Application WO 96/14830 (US Patent no 5,858,410) describes a drug carrier which comprises particles of a pure active compound which is insoluble or only sparingly soluble in water, which has an average diameter of 10nm to 1,000nm and the proportion of particles larger than 5 μ m in the total population is less than 0.1%. Preparation of the particles, with or preferably without surfactant, by means of cavitation (e.g. using a piston-gap
10 homogenizer) or by shearing and impact forces (i.e. the jet stream principle) is also described.

There is a need for a topical formulation of nanoparticulate spironolactone that overcomes the problems of formulating the drug for topical administration.

15

The applicants have now shown that for topical administration, the spironolactone in the form of nanoparticles can be successfully incorporated into a cream base consisting of a crystalline network of monoglycerides in water.

20

In a first aspect therefore the present invention provides a topical nanoparticulate spironolactone formulation comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of from about 300nm to about 900nm, preferably 400nm to 600nm incorporated
25 into a crystalline network system comprising a dispersion of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards the spironolactone nanoparticles.

The formulation is suitable for application to the skin for use in treating dermatological conditions known to be treatable with antiandrogens e.g. acne, androgenic alopecia, hirsutism and rosacea. Cream bases consisting of a crystalline network of monoglycerides are described in WO87/02582, WO82/03173 and WO93/20812. Examples of such crystalline networks of monoglycerides are known as Crystalip™.

The lipids may have a crystallisation temperature of between 20°C and 100°C. Preferable lipid crystals are β -crystals from a monoglyceride of a fatty acid having a chain length of 12-18 carbon atoms or monoglycerol ethers having ether chains of the corresponding length or fatty acid esters of ascorbic acid with a fatty acid chain length of 12-18 carbon atoms or mixtures thereof. The fatty acids as well as the ethers may be saturated or unsaturated, preferably saturated ones.

The fatty acids may therefore include lauric acid (C₁₂), myristic acid (C₁₄), palmitic acid (C₁₆) or stearic acid (C₁₈), although C₁₃, C₁₅, or C₁₇ acids could also be used.

Preferable monoglycerides may be a 1- or 2- monoglyceride, preferably a 1-monolaurin, 1-monomyristin, 1-monopalmitin and 1-monostearin or a mixture of two or more of these such as a mixture of 1-monolaurin and 1-monomyristin. Examples of unsaturated monoglycerides are monopalmitolein, monoolein, monolinolein and monoliniolenin.

The composition consists essentially of a dispersion of the above lipid crystals

in water or any other polar liquid or mixtures thereof having the ability to allow crystal formation. Examples of polar lipids for use in accordance with the invention are water, glycerol, propylene glycol and ethylene glycol or mixtures thereof, however other suitable polar lipids may also be used.

5

The spironolactone is protected within the network up to the time of use but upon application to the skin, the spironolactone comes into contact with the skin surface as a consequence of softening or melting of the crystalline structure of the shell.

10

Generally one would expect a noticeable increase in particle size on storage following the incorporation of very fine solid particles into a matrix which contains hydrophilic as well as lipophilic structures. Surprisingly, this did not happen and there was no noticeable crystal growth of Spironolactone over a seven month period. Furthermore, the cream has shown an increased flux rate in a membrane model with respect to a cream with non-nanoparticulate spironolactone.

15

As is well known in the pharmaceutical art, particle size may be measured by a variety of methods, which can give rise to apparently different reported particle sizes. Such methods include photon correlation spectroscopy (PCS) and laser diffraction. Furthermore the particle size may be reported as an average particle size (e.g. a number average, weight average or volume average particle size). In the present specification, unless indicated otherwise, the particle size will be quoted as a volume average particle size. Thus for example, a D_{50} of 500nm indicates that 50% by volume of the particles have a diameter of less than 500nm. Alternatively it can be stated that the particles having a diameter of less

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than 500nm occupy 50% of the total volume occupied by the total number of particles.

5 When the particle size of spironolactone according to the present invention is measured by laser diffraction the D_{50} is in the range 350-750nm and the D_{99} is in the range 500-900nm.

10 Nanosuspensions and nanoparticles comprising spironolactone according to the present invention preferably incorporate a stabiliser to prevent aggregation of the nanoparticles. Such stabilisers, which are well known in the art, are described in more detail hereinafter.

15 In this specification nanoparticles comprising spironolactone and nanosuspensions comprising spironolactone according to the present invention will be referred to as nanoparticulate spironolactone. It should be appreciated that this term also includes nanoparticles and nanosuspensions comprising spironolactone in association with a stabiliser.

20 Nanoparticulate spironolactone according to the invention, may be prepared by any known method for the preparation of nanoparticles, in particular by high pressure homogenisation.

25 The nanoparticulate spironolactone may be prepared by subjecting a coarse dispersion of spironolactone to cavitation. Preferably the nanoparticles are prepared using a high pressure piston-gap homogeniser. The nanoparticles may be associated with a stabiliser. Such stabilisers, which are well known in the art, are described in more detail hereinafter.

For the preparation of nanoparticles it is preferred that the spironolactone starting material be utilised in the form of coarse particles, preferably having a particle size of less than about 100 μ m. If necessary, the particle size of the spironolactone may be reduced to this level by conventional means, such as milling. The coarse particles of spironolactone are preferably dispersed in a liquid medium comprising a solvent in which the drug substance is essentially insoluble. In the case of spironolactone the liquid medium preferably comprises an aqueous solvent and most preferably consists essentially of water. The concentration of spironolactone in the said dispersion of coarse particles may be in the range 0.1 to 50%. The coarse dispersion may then be utilised in any known method for obtaining nanoparticles.

A preferred method is high pressure homogenization, wherein particle size is reduced mainly by cavitation. This is most preferably achieved using a high-pressure piston-gap homogeniser. In this method, the dispersion of coarse particles is forced at a high flow rate through a gap which is approximately 25 μ m wide. The static pressure exerted on the liquid falls below the vapour pressure of the liquid. The liquid therefore boils, resulting in the formation of gas bubbles within the area of the gap. However, once the liquid exits from the gap, normal pressure prevails and the gas bubbles collapse. The powerful implosion forces which result are strong enough to break up the coarse particles of drug substance, resulting in the formation of nanoparticles.

High pressure homogenisation may be carried out at a pressure in the range 100 to 3000 bar, preferably 1000 to 2000 bar (10^7 to 3×10^8 Pa, preferably 10^8 to 2×10^8 Pa) and at a temperature in the range 0 to 50°C, preferably 10 to 20°C,

e.g. around 15°C. The homogenisation may be carried out in a series of cycles until the desired particle size is obtained, or as a continuous process, e.g. over a period of 2-30 hours, preferably 2-10 hours.

5 Nanosuspensions of spironolactone according to the present invention preferably incorporate a stabiliser to prevent aggregation of the nanoparticles. Said stabiliser may be introduced at any suitable stage during the manufacture of the nanosuspension. Thus for example, surfactant may be added to the initial
10 the particles size, e.g. by high pressure homogenization, has taken place. Alternatively a portion of the stabiliser may be added before and a portion after the step of particle size reduction. Preferably stabiliser is present in the coarse dispersion. The concentration of stabiliser, either in the coarse dispersion or the nanosuspension may be in the range 0 to 10%.

15

Stabilisers which may be employed in the preparation of nanosuspensions according to the present invention may be selected from conventional stabilisers, and may include compounds which are also described as surfactants and surface modifiers. Thus examples of stabiliser which may be employed
20 include: polyoxyethylene sorbitan fatty acid esters, e.g. Tweens and Spans; polyoxyethylene stearates; polyoxyethylene alkyl esters; polyethylene glycols; block polymers and block copolymers such as poloxamers e.g. Lutrol F68, and poloxamines; lecithins of various origin (e.g. egg-lecithin or soya-lecithin), chemically-modified lecithins (e.g. hydrated lecithin), as well as phospholipids
25 and sphingolipids, sterols (e.g. cholesterol derivatives, as well as stigmasterin), esters and ethers of sugars or sugar alcohols with fatty acids or fatty alcohols (e.g. saccharose monostearate);

ethoxylated mono- and diglycerides, ethoxylated lipids and lipoids,
dicetyl phosphate, phosphatidyl glycerine, sodium cholate, sodium
glycolcholate, sodium taurocholate; sodium citrate;
cellulose ethers and cellulose esters (e.g. methyl cellulose, hydroxyethyl
5 cellulose, hydroxypropyl cellulose, sodium carboxymethyl cellulose), polyvinyl
derivatives such as polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyl acetate,
alginates, polyacrylates (e.g. carbopol), xanthanes; pectins, gelatin, casein, gum
acacia, cholesterol, tragacanth, stearic acid, calcium stearate, glyceryl
monostearate, dioctyl sodium sulfosuccinate (sodium docusate); sodium lauryl
10 sulfate, sodium dodecyl sulphate, benzalkonium chloride, alkyl aryl polyether
sulfonate, polyethylene glycols;
colloidal silicon dioxide, magnesium aluminium silicate; and phosphates.

A preferred stabiliser is sodium docusate, which is commercially available as a
15 70% solution in propylene glycol, under the name Octowet 70PGTM (sodium
dioctyl sulfosuccinate).

It will be appreciated from the foregoing that the process is carried out in a
liquid medium and hence the nanoparticulate spironolactone product is initially
20 obtained in the form of a nanosuspension. If desired the liquid medium may be
removed, e.g. by lyophilisation or spray drying to provide nanoparticulate
spironolactone in solid form. It will be appreciated that where a stabiliser is
present during the manufacture of a nanosuspension, the corresponding dried
nanoparticulate product will be associated with said stabiliser.

25

Following preparation of the nanoparticulate spironolactone, the formulation
according to the invention may be prepared as follows. The polar lipid is mixed

with water and/or any other polar liquid (such as glycerol, ethylene glycol or propylene glycol) having the ability to form crystalline network structures from polar lipids. The mixture formed has a concentration of water and/or polar liquid, respectively, of 50-95 percent by weight. The mixture is heated to a
5 temperature above the transition temperature of the lipid. The transition temperature is defined as the lowest temperature at which a particle of the lipid in contact with an excess of water or polar liquid absorbs water or polar liquid respectively and is converted into cylindrical or spherical crystalline structures having a strong birefringence. The mixture is maintained above the transition
10 temperature with stirring until the conversion has taken place. The mixture is then cooled with continued stirring to ambient temperature or the desired temperature, so that solid crystalline networks are formed. It is during this cooling down, at a temperature of about 30 to 35°C that the characteristic crystalline structure is formed.

15

The nanoparticulate spironolactone is dispersed in the mixture of polar lipid and water or polar liquid before or while the lipid is transformed into crystalline structures. To ensure that the nanoparticulate spironolactone is incorporated into the crystalline structure it must be added before the mixture is cooled
20 below 30 to 35°C.

25

If the nanoparticulate spironolactone is added after the mixture has been cooled to below 30 to 35°C, a physical mixture is formed but it does not form part of the crystalline structure. The nanoparticulate spironolactone therefore does not benefit from protection from and prevention of re-crystallisation and particle size growth of the active component since crystal layers are not formed around the active particles.

Nanosuspensions as used in the present invention in the formation of the topical formulation do not however respond well to heating. During heating, agglomerates may be formed and the active component may go into solution at higher temperatures. This can lead to re-crystallisation during the cooling down period which can result in a considerable increase in particle size.

The applicants have however determined a formulation, process and temperature of incorporation in order to allow formation of the crystalline structure after the addition of the nanosuspension, while keeping the heat exposure of the nanosuspension to a minimum.

Topical nanoparticulate spironolactone formulations according to the present invention advantageously incorporate the active drug in the form of a nanosuspension, most preferably in aqueous solution. Pharmaceutical formulations according to the present invention may be prepared according to methods well known in the art.

Topical formulations according to the present invention may be provided as an ointment, cream, gel, liquid, spray or mousse. Aqueous preparations may contain the nanosuspension as such; non-aqueous preparations can alternatively comprise dried nanoparticles.

In a second aspect the present invention provides a topical nanoparticulate spironolactone formulation for use in the topical treatment of conditions known to be treatable with antiandrogens, e.g. acne, androgenic alopecia, hirsutism and rosacea.

In a third aspect, the invention provides the use of spironolactone nanosuspensions comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of from about 300nm to about 900nm, preferably 400nm to 600nm in the manufacture of a medicament for the treatment of a condition responding to antiandrogens, such as acne, hirsutism, androgenic alopecia or rosacea. The medicament may be adapted for topical application. The nanoparticles may be incorporated into a cream base which may consist of a crystalline network of monoglycerides in water or other polar liquids.

This aspect of the invention extends to providing a method of treating a condition responding to anti androgens comprising administering nanoparticulate spironolactone formulation as defined above to a patient in need of such treatment. The condition may be acne, hirsutism, androgenic alopecia or rosacea.

In a fourth aspect, the invention provides preparations comprising crystalline network system of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards an incorporated substance for use in the topical treatment of acne. The crystalline network system of solid crystals of polar lipids have previously been referred to as microcapsules in WO 87/02582.

In a fifth aspect there is provided a process for the preparation of a topical nanoparticulate spironolactone formulation comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of

from 300nm to about 900nm, wherein the process comprises incorporation of a nanosuspension of spironolactone into an aqueous dispersion of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards the spironolactone nanoparticles.

5

The nanosuspension may be incorporated when the mixture has been cooled to between 60°C and 35°C, more preferably 55°C to 45°C, suitably 50°C before the mixture reaches its crystallisation point. The temperature of the nanosuspension at incorporation is preferably equal to room temperature i.e. 20 to 25 °C.

10

Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

15

The invention will now be illustrated with reference to one or more of the following non-limiting examples and figures.

20

Figure 1 relates to a microscope picture of nanoparticulate spironolactone according to the present invention immediately after it has been prepared. The scale relates to a distance between each bar of 0.01mm.

Figure 2 relates to a microscope picture of nanoparticulate spironolactone according to the present invention after 7 months storage at room temperature. The scale relates to a distance between each bar of 0.01mm.

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Figure 3 relates to a microscope picture of commercially available spironolactone in non-nanoparticulate form. The scale relates to a distance between each bar of 0.01mm.

Figure 4 relates to a typical calibration curve of Spironolactone standards of

0.93-59.2 µg/ml.

Figure 5 relates to a graph showing the mean flux of spironolactone from the nanosuspensions (2%w/w) and aqueous cream (2% w/w) (n=4, mean +/- SE)

$y = 35.552x - 20.258$ $r^2 = 0.991$ Spironolactone aqueous cream 2%

5 $y = 42.097x - 26.784$ $r^2 = 0.989$ Spironolactone nanosuspension 2%

Figure 6 relates to *S. epidermidis* mean zone diameter of Crystalip™ spironolactone formulation compared to 2%w/w spironolactone in Aqueous cream B.P. (mean ±S.D.; n=5)

10 Figure 7 relates to *P. acnes* mean zone diameter of Crystalip™ spironolactone formulation compared to 2% spironolactone w/w in Aqueous cream B.P. as comparator (mean ± S.D; n=5).

Figure 8 relates to the particle size of spironolactone nanosuspension following heating to 50°C (■) or 70°C(□) then cooling compared to unheated nanosuspension (▣)

15 Figure 9 relates to the particle size of spironolactone nanosuspension 24 hours after heating to 50°C (■) or 70°C (□) then cooling compared to unheated nanosuspension (▣)

Figure 10 relates to the viscosity of the mixture following introduction of Octowet™ at 50°C, 70°C or room temperature.

20 Figure 11 relates to the effect of the composition of the mixture on its viscosity.

Examples

Example 1: Preparation of nanoparticulate spironolactone as a topical formulation.

25

Preparation of nanoparticulate spironolactone.

| Formulation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------------------|------|------|------|------|------|------|------|------|-------|
| Sample volume (ml) | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 100 | 500 |
| Results | | | | | | | | | |
| D ₅₀ (micron) | 1.69 | 0.85 | 1.06 | 0.84 | 0.88 | 0.86 | 0.78 | 0.54 | 0.539 |
| D ₉₀ (micron) | 4.39 | 1.83 | 2.49 | 1.92 | 1.82 | 1.5 | 1.8 | 0.68 | 0.772 |
| PCS mean diameter | - | 581 | 880 | 608 | 681 | 656 | 609 | 415 | 436 |
| PI | - | 0.7 | 0.2 | 0.15 | 0.03 | 0.1 | 0.2 | 0.05 | 0.1 |

Preparation of a Crystalip™ composition

Table 2 illustrates representative preparations of nanoparticulate spironolactone as a topical cream, using formulations 7, 8 or 9 as shown in Table 1. The topical nanosuspension preparations were prepared as follows:

Water was heated to 70°C and propylene glycol added. The monoglycerides were melted at 70°C, and the molten monoglycerides were then added to the water phase under stirring at 70rpm. Cooling down of the mixture was then started. The stirring speed was increased to 95rpm when the mixture reached around 50°C when the viscosity increased and the cold nanosuspension added. The stirring speed was decreased to 75rpm at 35°C when the β -crystalline structure started to form.

Table 2

| Formulation | A | B | C | D |
|--|----|----|----|----|
| Spironolactone Nanosuspension (formulations 7, 8 or 9 from table 1) | 20 | 10 | 20 | 20 |
| Glycerine monolaurate | 7 | 6 | 6 | 5 |
| Glycerine monomyristate | 21 | 18 | 18 | 15 |
| Propylene glycol | -- | 10 | 10 | 20 |
| Water | 52 | 56 | 46 | 40 |

5 The following experiments were performed to determine the optimum compositions and method for producing a topical nanoparticulate spironolactone formulation in accordance with the present invention.

Selection of an optimum Crystalip™ composition.

10 Two batches of a Crystalip™ composition were produced as shown in the table below.

| Batch number | Glycerine monolaurate | Glycerine monomyristate | Propylene glycol (PG) | Water |
|--------------|-----------------------|-------------------------|-----------------------|-------|
| 4011-001/01 | 7% | 21% | / | 72% |
| 4011-001/02 | 5% | 15% | 20% | 60% |

Table 3: Reproduction of Crystalip™ placebo

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For the first batch, a characteristic exothermic peak was seen during cooling. At the end of the cooling stage the cream was very viscous but did not have the shiny appearance typical of a β -crystalline structure. During storage, this shiny appearance started to appear.

20

For the second batch, an exothermic peak was not observed, however there was a shiny appearance. The viscosity seemed to be lower than the first batch.

A composition with less propylene glycol was then tested (table 4) since there were some concerns about irritation resulting from high PG concentrations. Propylene glycol is useful to retain in the composition since it may increase the antimicrobial efficacy of the base and enhance penetration of active components into the skin therefore. The following batches were manufactured:

| Batch number | Glycerine monolaurate | Glycerine monomyristate | Propylene glycol (PG) | Water |
|--------------|-----------------------|-------------------------|-----------------------|-------|
| 4011-001/03 | 5% | 15% | 10% | 70% |
| 4011-001/04 | 6% | 18% | 10% | 66% |

Table 4: Crystalip™ batches

For the batch with 20% monoglycerides (MG), viscosity dropped at around 40°C, and the mixture became liquid again just after the viscosity had started to increase.

The MG level was therefore increased to 24%. The viscosity also dropped around 40°C, but viscosity increased again during further cooling. An exothermic peak was observed at 33°C and the final cream had a shiny appearance, which means the final β -crystalline structure had been produced.

Compatibility tests between the surfactant and Crystalip™.

It had previously been determined that Crystalip™ is compatible with Myrj 59 and Span 20, which are both non-ionic surfactants. However, Octowet 70PG is used for the spironolactone nanosuspension, which is an anionic surfactant. Octowet 70PG is also known as Sodium dioctyl sulfosuccinate and is a 70% solution (70% DOSS) of water and propylene glycol

For a cream containing 2% spironolactone, 20% of the spironolactone nanosuspension must be incorporated since the nanosuspension contains 10% active spironolactone and 0.5% surfactant. In testing the compatibility of the Crystalip™ formulation with Octowet solution, 20% Octowet solution was therefore used and replaced part of the water phase.

The compatibility of Octowet 70PG solution with the chosen Crystalip™ formulation (10%PG + 24%MG) was tested. Three batches of Crystalip™ (table 5) were manufactured in which 20% of a 0.5% Octowet 70PG solution were introduced at different temperatures: 70°C, 50°C and room temperature.

| Batch number | Glycerine mono laurate | Glycerine mono myristate | Propylene glycol | Octowet solution (0.5%) | Water | T °C * |
|--------------|------------------------|--------------------------|------------------|-------------------------|-------|-----------|
| 4011-001/05 | 6% | 18% | 10% | 20% | 46% | 70 |
| 4011-001/06 | 6% | 18% | 10% | 20% | 46% | 50 |
| 4011-001/07 | 6% | 18% | 10% | 20% | 46% | room temp |

Table 5: Crystalip™ batches including Octowet solution

*T °C: temperature at which the surfactant solution has been introduced.

15

All three batches produced the exothermic peak and resulted in a shiny appearance. The viscosity of the batches number 05 and 06 was acceptable, but the viscosity of batch number 07 after introduction of the Octowet solution was not sufficient.

20

It was therefore concluded that the chosen Crystalip™ formula was compatible with 20% of a 0.5% Octowet solution. The Octowet solution was best

incorporated at a temperature above the crystallisation point, rather than at room temperature.

Introduction of spironolactone nanosuspension in Crystalip™

5

The composition of the nanosuspension was 10% spironolactone and 0.5% Octowet 70PG.

10

Particle size of the fresh nanosuspension using laser diffraction (COULTER):D50 = 0.686

D90 = 1.033

D99 = 1.033

Particle size using photon correlation spectroscopy (Zetasizer 3000HS): Z average = 476nm

15

From the compatibility tests above it was known that incorporating materials at room temperature was not a good option. It was therefore necessary to investigate heating the nanosuspension. The particle size before and after heating the suspension was measured.

20

The experiment was carried out as follows:

Two samples of nanosuspension were heated to 50°C and 70°C, respectively. The temperature was held for 10 min and then the sample was cooled back to room temperature. The particle size was measured by photo correlation spectroscopy and laser diffraction at these time points:

25

- after ultrasonication, before heating (reference)
- after cooling down (t0)
- after 24h (t24h)

| Particle size distribution | Sample identity | | | | |
|----------------------------|-----------------|-----------|-----------|-------------|-------------|
| | Reference | 50°C - t0 | 70°C - t0 | 50°C - t24h | 70°C - t24h |
| D50 | 0.579 nm | 0.638 nm | 0.700 nm | 0.634 nm | 0.694 nm |
| D90 | 0.740 nm | 0.878 nm | 1.053 nm | 0.861 nm | 1.572 nm |
| D99 | 0.850 nm | 1.922 nm | 1.421 nm | 1.902 nm | 38.65 nm |

Table 6: Determination of size particles by laser diffraction (Coulter)

The results of Table 6 are also shown in Figures 8 and 9.

5

| Sample identity | Reference | 50°C - t0 | 70°C - t0 | 50°C - t24h | 70°C - t24h |
|---------------------|-----------|-----------|-----------|-------------|-------------|
| Particles size (nm) | 456.3 | 469.7 | 538.3 | Not done* | Not done* |

Table 7: Determination of particle size by photon correlation spectroscopy (Zetasizer 3000HS)

(*): the size was found too large by laser diffraction measurement with PCS was not made

10

It was shown that the results from heating the nanosuspension show a quite dramatic particle size increase for 70° C, particularly after waiting for another 24h, which suggests some drug had gone into solution and then re-crystallised. The particle size also increased at 50° C, however there did not seem to be so much recrystallisation happening over the following 24 hours.

15

It was decided that a very short exposure to 50° C would be acceptable. It was therefore concluded that the final batch should be prepared as follows:

20

Crystalip™ with a reduced water phase would be prepared. The nanosuspension would be sonicated, but not heated. Once the temperature of the Crystalip™ reached 50° C, the cold nanosuspension would be added, leading to a quick temperature decrease and minimisation of heat exposure of the spironolactone suspension. This process would also not interfere with Crystalip™ crystallisation, which occurs at lower temperatures.

A batch was prepared according to the above recommendation where cold nanosuspension was added to Crystalip™ at 50° C (table 8).

| Batch number | Glycerine mono laurate | Glycerine mono myristate | Propylene glycol | Nano suspension | Water |
|---------------|------------------------|--------------------------|------------------|-----------------|-------|
| 4011-001/08ac | 6% | 18% | 10% | 20% | 46% |

Table 8: Crystalip™ batches including spironolactone nanosuspension

The batch was successful. The final cream had a good viscosity and a shiny, homogenous appearance.

Viscosity measurement

| Sample ID | Composition |
|---------------|---|
| Bioglan | 20%MG-20%PG |
| 4011-001/05pc | 24%MG-10%PG-20%Octowet solution (introduce at 70°C) |
| 4011-001/06pc | 24%MG-10%PG-20%Octowet solution (introduce at 50°C) |
| 4011-001/07pc | 24%MG-10%PG-20%Octowet solution (introduce at room temperature) |
| 4011-001/08ac | 24%MG-10%PG-20%nanosuspension |
| 4011-001/13pc | 28%MG |
| 4011-001/17pc | 20%MG-20%PG |

The results of the viscosity results of the compositions shown in the above table are shown in Figures 10 and 11.

Tests with the second batch of nanosuspension : 3011-05an1

5

As the nanosuspension was not fresh for the previous tests it was decided to make a new nanosuspension of spironolactone and to incorporate it in Crystalip™ just a few days after the manufacture. The composition of the nanosuspension used was 10% spironolactone and 0.5% Octowet 70PG. It was also investigated whether it was possible to introduce 30% of nanosuspension instead of 10% in the Crystalip™.

10

The particle size of the nanosuspension, just after the making, using laser diffraction was as follows:

15 (COULTER): D50 = 0.443

D90 = 0.657

D99 = 0.738

Particle size using photon correlation spectroscopy (Zetasizer 3000HS): Z average \approx 419.3 nm

20

Two new batches of Crystalip™, 4014-000/01ac and 014-000/02ac were made with respectively 20% and 30% of nanosuspension in it. The process was identical to 4011-001/08ac (incorporation of the nanosuspension at 50°C during the cooling stage). The nanosuspension was manufactured the day before the making of Crystalip™. For those two batches the pH was adjusted to the same as the market cream (Spiroderm 5% with a pH= 4,16). The two batches are summarised in the following table.

25

| Batch number | Glycerine mono laurate | Glycerine mono myristate | Propylene glycol | 3011-05an1 | Water | Citric acid | Sodium Hydroxide |
|---------------|------------------------|--------------------------|------------------|------------|-------|-------------|------------------|
| 4014-000/01ac | 6% | 18% | 10% | 20% | 45.5% | 0.5% | Up to pH=4,16 |
| 4014-000/02ac | 6% | 18% | 10% | 30% | 35.5% | 0.5% | Up to pH=4,16 |

5 The two creams were shiny but the second one 4014-000/02ac seemed to have a higher viscosity. As the nanosuspension is introduced cold, the viscosity increases more quickly for the batch with 30% of nanosuspension. Moreover the batch with 30% of nanosuspension seemed to be less homogeneous because of the increase of the viscosity.

10 The pH and the density of those batches were measured the next day after manufacturing, as shown in the table below.

| Batch number | pH | Density (g/cm3) |
|---------------|------|-----------------|
| 4014-000/01ac | 4.29 | 0.989 |
| 4014-000/02ac | 4.22 | 0.984 |

Example 2: Size of spironolactone particles before and after storage.

15

Figures 1, 2, and 3 show microscope pictures of Spironolactone according to the invention immediately after preparation, after 7 months storage and their comparison to a commercial Spironolactone. The figures contain a scale which relates to a distance between each vertical bar of 0.01mm or 10 micrometres.

20

The particles shown in Figures 1 and 2 are almost too small to see in the light

microscope. There is no particle growth over 7 months storage. In contrast, the commercial spironolactone "Spiroderm" (Figure 3) has Spironolactone crystals present of up to 20 micrometres in size.

5 Example 3: Flux studies

The flux through artificial membranes of spironolactone (2%) from a nanosuspension formulation incorporated into "Crystalip™" matrix was measured in a Franz cell set-up and compared with 2% w/w spironolactone in Aqueous cream B.P. as a comparator.

| Material | Supplier |
|---|------------------------------|
| Crystalip™ spironolactone nanosuspension 2% Lot no. 4014-000/06atc | SkyePharma, Switzerland |
| Spironolactone Lot no. 510/0 | |
| Aqueous cream B.P. Lot no. 28076 | Hillcross, UK |
| Ethanol AnalaR grade | VWR International Ltd., UK |
| Sodium dihydrogen phosphate dihydrate Lot no. L298 | Merckeurolab |
| Deionised water | Elga Ltd., UK |
| Acetonitrile HPLC grade | Rathburns Chemicals Ltd., UK |
| Regenerated cellulose Membrane | NBS-Biological, UK |

Methods for flux studies

15

The Crystalip™ formulation was supplied by SkyePharma AG. A commercial Spironolactone comparator was not available any more at the time of the experiments. Therefore a comparator in a standard cream matrix was prepared as follows. Briefly, 100 mg of Spironolactone powder was accurately weighed

and mixed with 4.90 g Aqueous cream B.P. in order to obtain a 2% w/w non-nanoparticulate spironolactone in Aqueous cream formulation.

In-vitro diffusion studies

5

Ethanol: Phosphate 'buffer' (20:80 v/v, pH 4.5) was used as the receiver fluid in order to maintain stability of spironolactone and sink conditions. The artificial membrane used was regenerated cellulose membrane.

10 Franz cell diffusion studies

Individually calibrated Franz diffusion cells with an average diffusional surface area of 0.56 ± 0.03 cm² and an average receiver volume of 1.83 ± 0.02 ml were used to conduct the diffusion experiments. The Spectra/Por® cellulose
15 membranes were cut to appropriate size and immersed in deionised water for 30 min to remove the preservative (0.1% sodium azide), wiped with tissue to remove surface liquid and mounted onto the Franz cells. The receiver fluid was incorporated into the Franz cell, stirred constantly with a magnetic stirrer and maintained at 32°C. The membranes were allowed to equilibrate with the
20 receiver phase for 30 min before applying the formulations. Each formulation (200 µl) was applied onto the membrane surface using a positive displacement Finnpiptette®. Five sampling times were investigated (1, 2, 4, 6 and 8 h) whereby 200 µl of the receiver fluid was carefully withdrawn from the arm of the Franz cell; each sample removed was replaced by an equal volume of fresh
25 pre warmed (32°C) receiver fluid. Throughout the experiment, any losses in receiver fluid due to evaporation from the Franz cell were replaced to maintain

a constant volume. Samples were analysed via HPLC using chromatographic conditions as follows:

| | | |
|----|---------------------|--|
| 5 | Column: | Hypersil 3 μ m Phenyl BDS column (s/no. 182862) |
| | Column length: | 150 x 4.60 mm |
| | Column temperature: | 30°C |
| | Mobile phase: | 50mM Phosphate buffer:acetonitrile (70:30 v/v) |
| | Flow rate: | 1.0 ml/min |
| 10 | UV wavelength: | 238 nm |
| | Injection volume: | 10 μ l |
| | Run time: | 15 min |

Preparation of spironolactone standard curves

15

Spironolactone standards were prepared in receiver fluid and calibration curves were constructed in the range 0.93 – 59.2 μ g/ml. Calibration curves with $r^2 > 0.999$ were considered acceptable and a typical curve is illustrated in Figure 4.

20

Data analysis

25

The amount of spironolactone in the receiver fluid was corrected for sample removal. The cumulative amount of spironolactone permeated per unit membrane surface was plotted against the square root of time and the slope of the linear portion of the graph was estimated as the steady state flux. A Student's t-test was employed to statistically determine any significant

difference in release of spironolactone from the 2% spironolactone nanosuspensions and 2% spironolactone Aqueous cream..

Results

5

Figure 5 shows a graphical representation of the mean cumulative amount of spironolactone permeated per unit area ($\mu\text{g}/\text{cm}^2$) from the two spironolactone formulations. These profiles show steady state flux for both formulations. The release rate of spironolactone from the spironolactone nanosuspension (2% w/w) was shown to be significantly faster than the aqueous cream formulation ($p=0.08$).

10

Example 4: Zone of inhibition assay

The antimicrobial action of two spironolactone formulations, namely spironolactone nanosuspension 2% w/w and spironolactone coarse 2% w/w, and their respective placebos were compared. A 2% w/w spironolactone in Aqueous cream B.P. formulation (in-house) was used as a comparator. The following materials were used.

20

| Material | Supplier |
|---|--------------------------------------|
| <i>Staphylococcus epidermidis</i> (ATCC 12228) | Oxoid Ltd, UK. |
| <i>Pseudomonas acne</i> (NTC 737) | Central Public Health Laboratory, UK |
| S&S Antibiotic-Assay discs(filter paper), diam ¼ inch | Aldrich Chemical company, USA |
| Aqueous cream B.P. Lot no. 28076 | Hillcross, UK |
| Spironolactone Lot no. 510/0 | SkyePharma, Switzerland |

Methods

The Crystalip™ spironolactone and placebo formulations were prepared as shown in the table below. Since a commercial Spironolactone comparator was not available any more, a comparator in a standard cream matrix was prepared as follows. Briefly, 100 mg of Spironolactone powder was accurately weighed and mixed with 4.90 g Aqueous cream B.P. to obtain a 2% w/w non-nanoparticulate spironolactone in Aqueous cream.

The batch size is 500g, manufactured with a lab reactor IKA-LR1000.2.

| Composition | 4014-000/06atc Crystalip™ nanosuspension 2% |
|--------------------------------|---|
| MG (monoglycerides) | 24 |
| PG (propylene glycol) | 10 |
| Nanosuspension - 10% spiro | 20 |
| Spironolactone coarse | / |
| Octowet solution 0.5% w/w | / |
| Citric acid | 0.1 |
| Sodium Hydroxide up to pH 4.16 | |
| Water PPI | 45.9 |
| Physical properties | |
| pH | 4.31 |
| Density (g/ccm) | 0.900 |
| Viscosity (cP) | |
| 20 rpm | 42'327 |
| 50 rpm | 23'780 |

20 ± 1 mg of each of the samples listed below were carefully transferred onto the surface of ¼ inch antibiotic assay discs.

- 1 SkyePharma AG, Crystalip™, Spironolactone nanosuspension 2% w/w
4014-000/06atc
- 2 2% w/w Spironolactone in Aqueous cream BP

5

The antibiotic discs coated with each of the formulations were placed onto the surface of the organism seeded agar plates using a pair of sterile forceps.

The *S. epidermidis* plates were incubated for 24 h at 37°C.

10

The *P. acne* plates were incubated in the anaerobic jars and incubated for 72 h.

Zones of inhibition were measured using a pair of calipers.

15 Results

Figures 6 and 7 show the mean zone of inhibition for both formulations using seeded *S. epidermidis* and *P. acnes* plates, respectively. The Crystalip™ formulation with Spironolactone nanosuspension exhibited a considerable effect against those acne-related bacteria. Aqueous cream B.P. with 2% w/w spironolactone (comparator) showed no zones of inhibition.

20

The base matrix therefore adds an antibacterial effect (on *S. epidermidis* and *P. acnes*) to the formulation, which is not due to the Spironolactone. The formulation does not contain any further antibiotics, or preservatives. The comparator product, which shows zero antibiotic effect on these acne-related microorganisms is preserved with phenoxyethanol. The applicants product may

25

therefore improve acne through both the hormone activity of the drug and the antibacterial efficacy of the matrix in which it is contained.

5

10

CLAIMS

1. A topical nanoparticulate spironolactone formulation comprising
5 nanoparticles having a mean diameter, measured by photon correlation
spectroscopy, in the range of from about 300nm to about 900nm incorporated
into a crystalline network system comprising a dispersion of solid crystals of
polar lipids, said lipids exposing their hydrophilic side outwards and their
hydrophobic side inwards towards the spironolactone nanoparticles.
- 10
2. A formulation according to claim 1 comprising nanoparticles having a
mean diameter, measured by photon correlation spectroscopy, in the range of
from about 400nm to about 600nm.
- 15
3. A formulation according to claims 1 or 2 wherein the lipid has a
crystallisation temperature of between 20°C and 100°C
4. A formulation according to claims 1 to 3 wherein the lipid crystals are β
crystals of a monoglyceride of a fatty acid having 12-18 carbon atoms, or
20 ascorbic, phosphate or lactic esters of fatty acids or of monoglycerol ethers or
mixtures thereof.
5. A formulation according to claim 4 wherein the monoglyceride is 1-
monolaurin, 1-monomyristin, 1-monopalmitin or 1-monostearin or a mixture of
25 two or more of these.

6. A formulation according to claims 1 to 5 wherein the wherein crystalline network structures of polar lipids are formed within a polar liquid.
7. A formulation according to any of claim 6 wherein the polar liquid is selected from the group comprising water, glycerol, ethylene glycol or propylene glycol or mixtures thereof.
8. A topical nanoparticulate spironolactone formulation according to any of claims 1 to 7 for use in the topical treatment of acne, hirsutism, androgenic alopecia or rosacea.
9. A formulation according to claims 1 to 8 wherein the active drug is incorporated in the form of a nanosuspension.
10. A formulation according to claim 9 wherein the nanosuspension is an aqueous nanosuspension.
11. A formulation according to claim 10 wherein the nanosuspension comprises a stabiliser.
12. A formulation according to claim 11 wherein the stabiliser is sodium docusate.
13. Use of spironolactone nanosuspensions comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of from about 300nm to about 900nm in the manufacture of a medicament for the treatment of a condition responding to anti-androgens.

14 Use according to claim 13 wherein the condition is selected from acne, hirsutism, androgenic alopecia or rosacea.

5 15. Use according to claims 13 or 14 wherein the medicament is adapted for topical application.

16. Use according to claims 13 to 15 wherein the nanoparticles are incorporated into a cream base.

10

17. Use according to claim 16 wherein the cream base consists of a crystalline network of monoglycerides in water or other polar liquids.

15 18. A method of treating a condition responding to anti androgens comprising administering a nanoparticulate spironolactone formulation according to claims 1 to 6 to a patient in need of such treatment.

19. A method according to claim 18 wherein said condition is selected from the group consisting of acne, hirsutism, androgenic alopecia or rosacea.

20

20. A crystalline network system comprising a dispersion of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards an incorporated substance for use in the topical treatment of acne.

25

21. A process for the preparation of a topical nanoparticulate spironolactone formulation comprising nanoparticles having a mean diameter, measured by photon correlation spectroscopy, in the range of from 300nm to about 900nm, wherein the process comprises incorporation of a nanosuspension of spironolactone into an aqueous dispersion of solid crystals of polar lipids, said lipids exposing their hydrophilic side outwards and their hydrophobic side inwards towards the spironolactone nanoparticles.
- 5

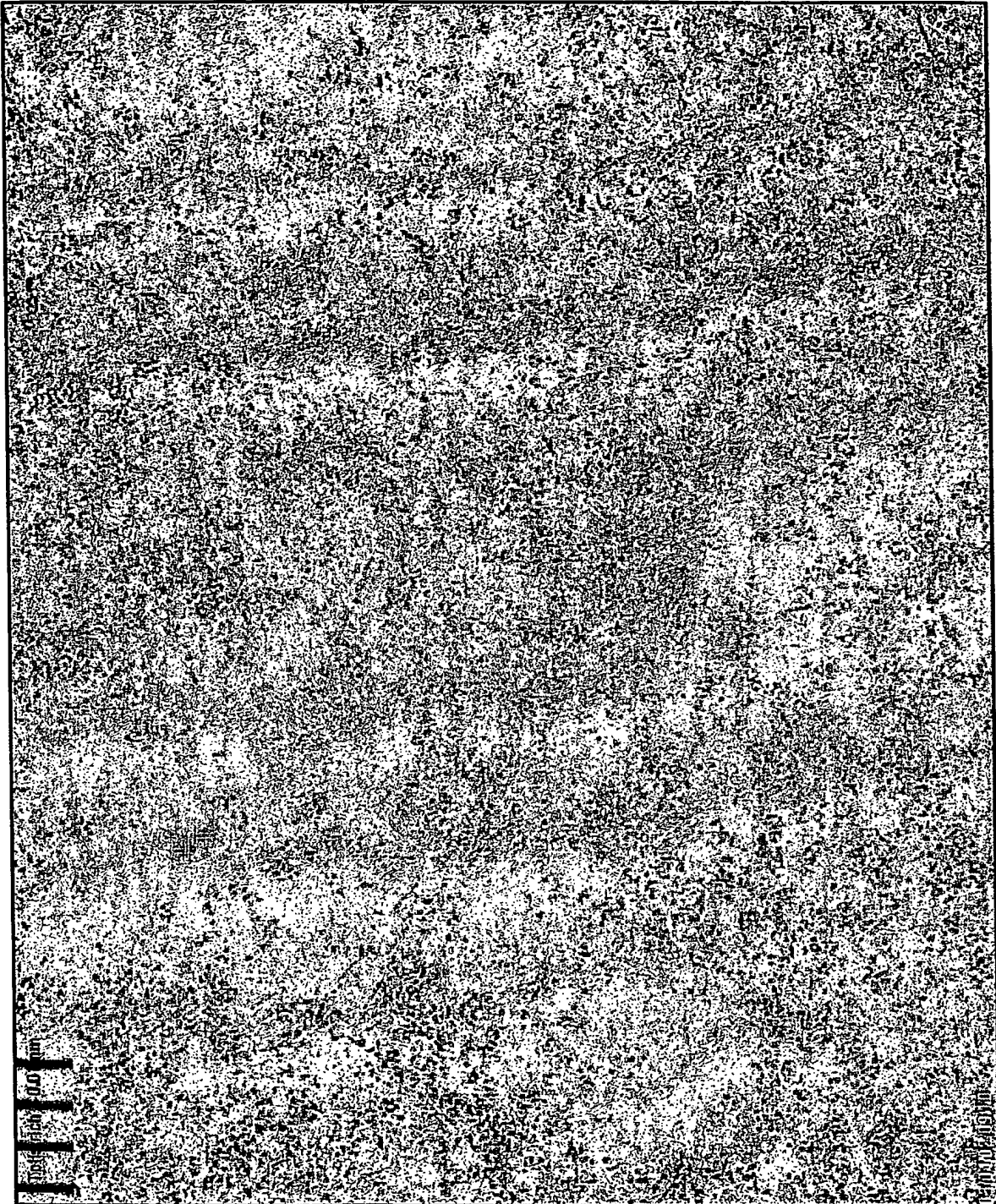


FIG. 1

2 / 7

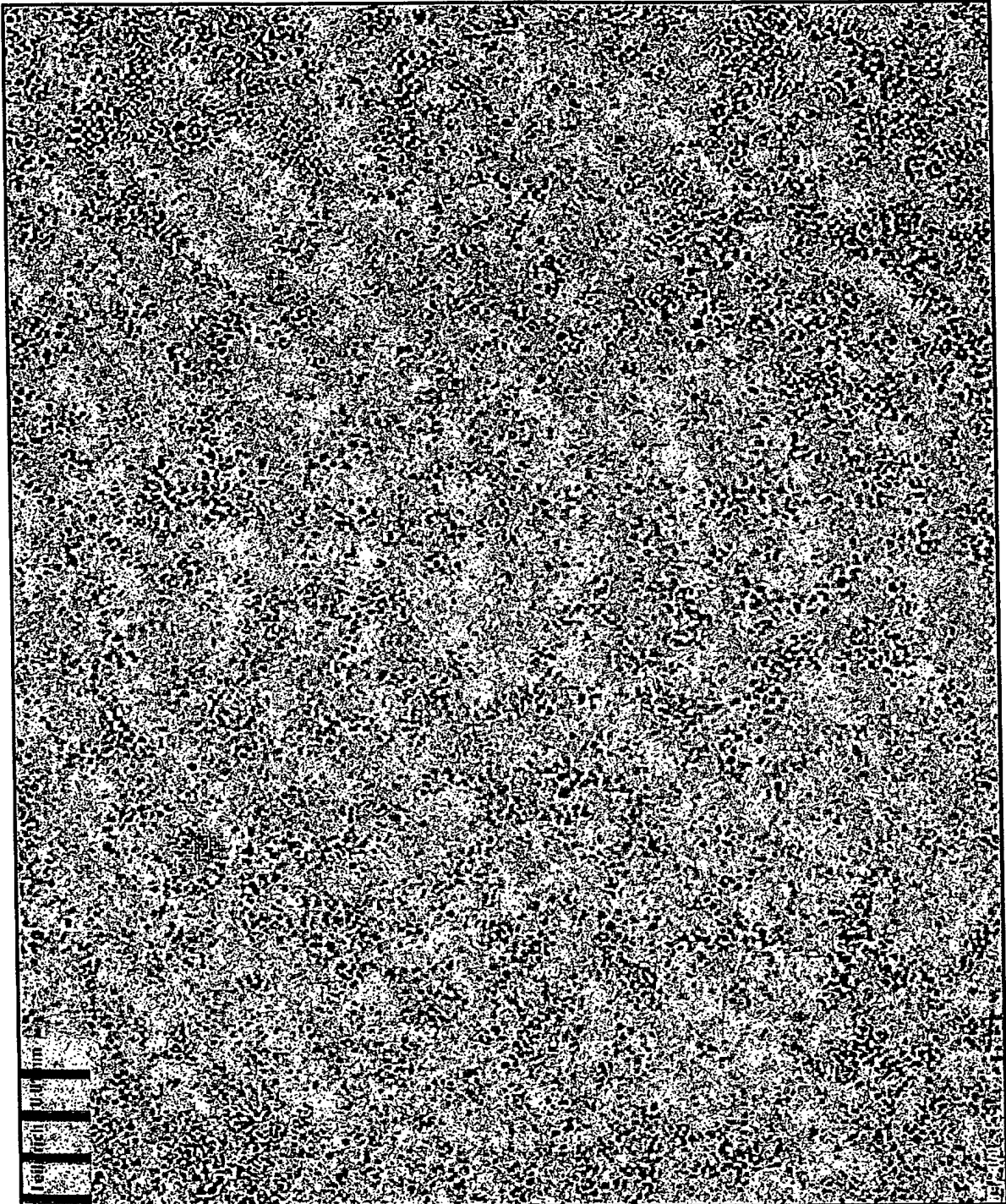


FIG. 2



FIG. 3

4 / 7

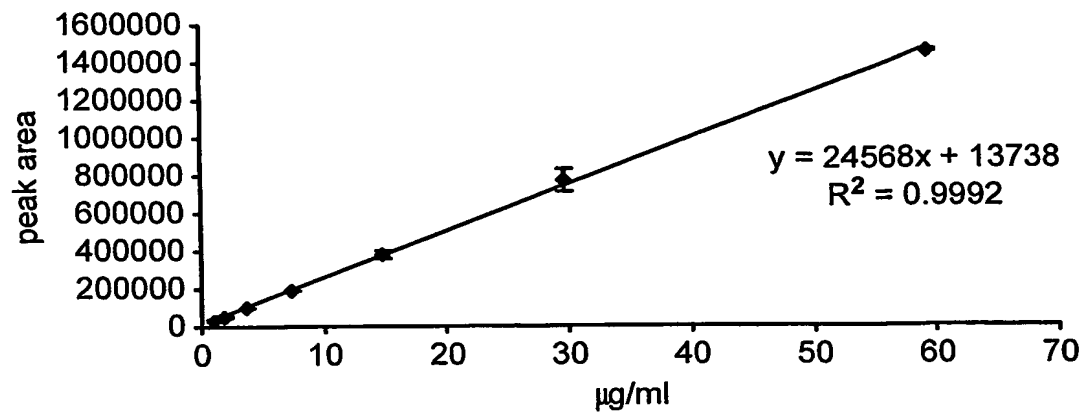


FIG. 4

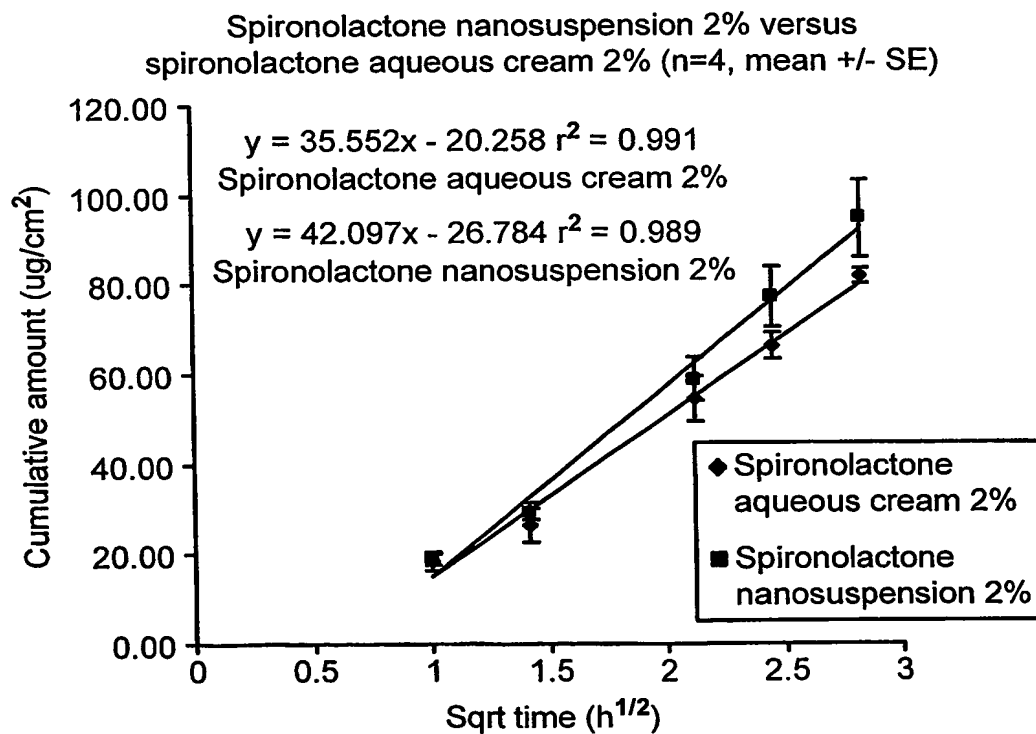


FIG. 5

5 / 7

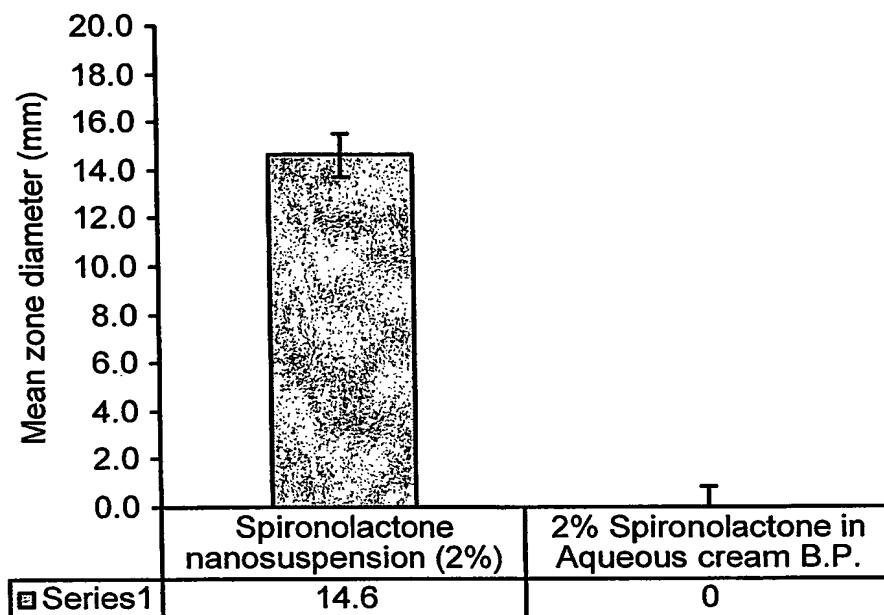


FIG. 6

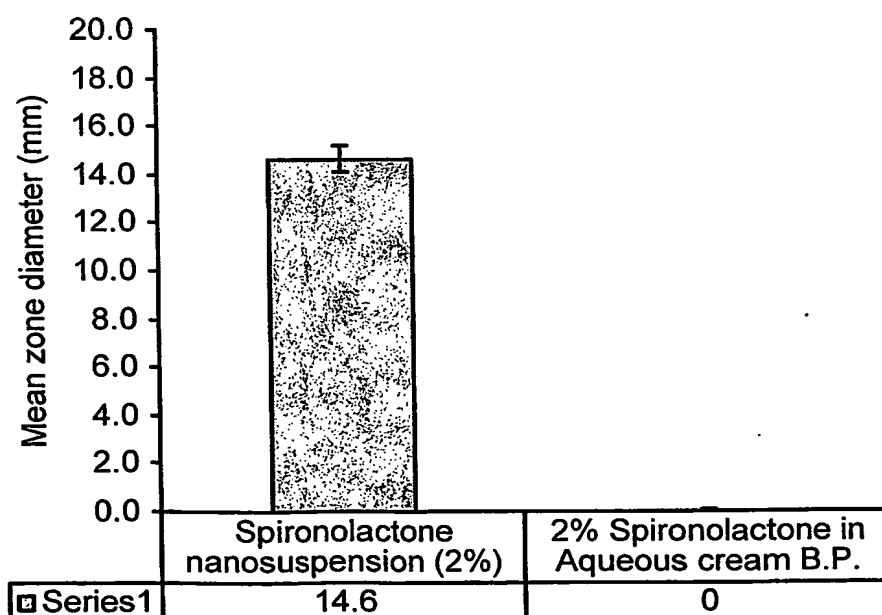


FIG. 7

6 / 7

Particle size of spironolactone nanosuspension

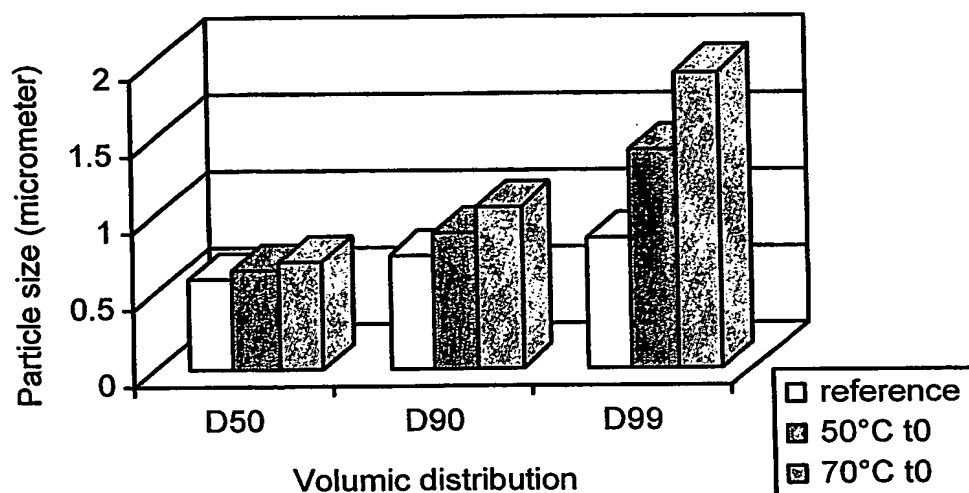


FIG. 8

Particle size 24h after heating

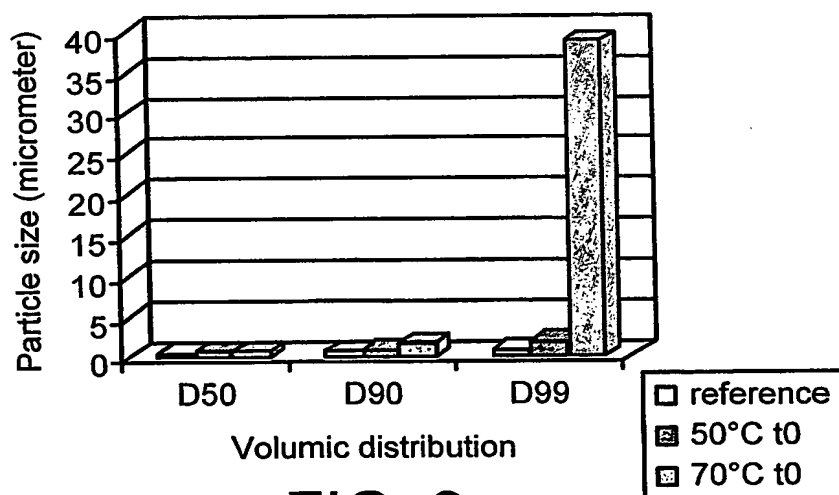


FIG. 9

7 / 7

Viscosity test report-Crystalip+Octowet solution

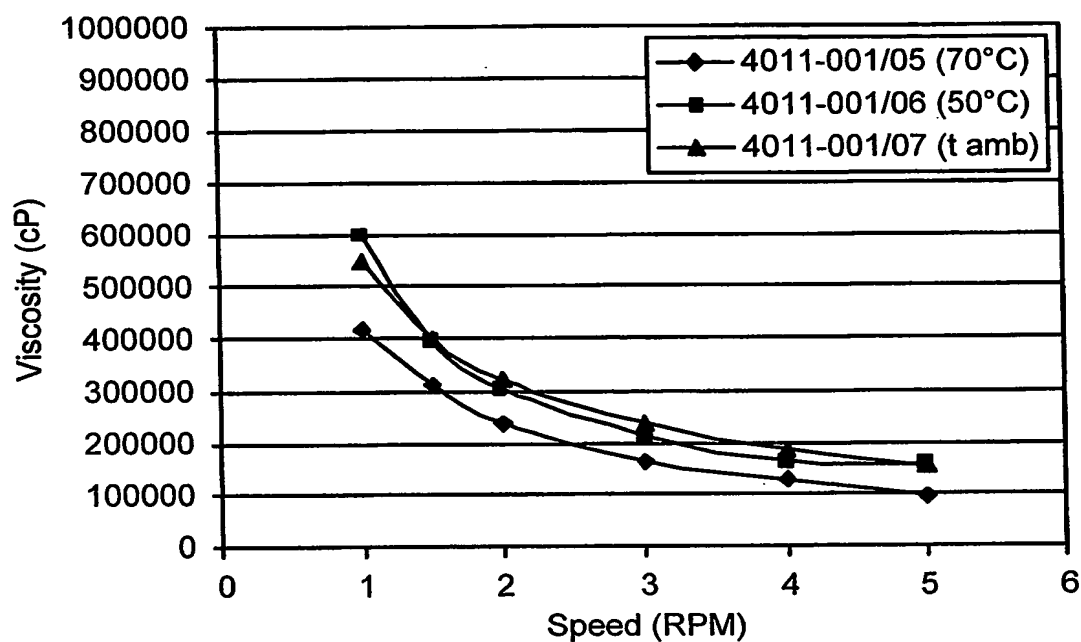


FIG. 10

Viscosity test report

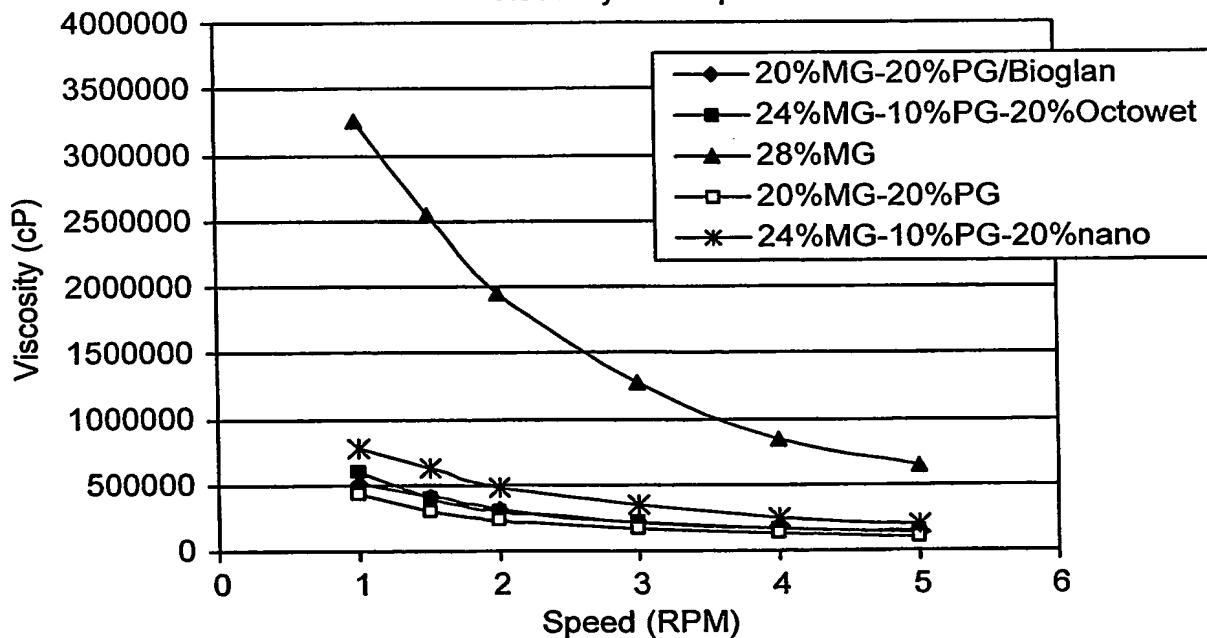


FIG. 11

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/05680

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K9/51 A61K9/06 A61K47/14 A61K31/585

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | WO 99 21534 A (MERCK) 6 May 1999 (1999-05-06) claims examples page 4, line 10 - line 13 | 1-21 |
| A | EP 0 256 285 A (BEHRINGWERKE) 24 February 1988 (1988-02-24) claims examples 4,5 | 1-21 |
| A | WO 83 00294 A (ARTHUR D. LITTLE INC., U.S.A) 3 February 1983 (1983-02-03) claims page 3, line 10 | 1-21 |
| -/-- | | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

16 July 2003

Date of mailing of the international search report

30/07/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Scarponi, U

INTERNATIONAL SEARCH REPORT

International Application No

PCT/02/05680

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | WO 87 02582 A (BIOGRAM) 7 May 1987 (1987-05-07) cited in the application claims ---- | 1-21 |
| A | US 5 091 188 A (D.H.HAYNES) 25 February 1992 (1992-02-25) cited in the application claims ---- | 1-21 |
| A | US 5 145 684 A (G.G.LIVERSIDGE ET AL.) 8 September 1992 (1992-09-08) cited in the application claims ---- | 1-21 |
| A | US 5 858 410 A (R.H.MULLER ET AL.) 12 January 1999 (1999-01-12) cited in the application claims ----- | 1-21 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/05680

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 18-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

on patent family members

International Application No

PCT/GB 02/05680

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|--|---|------------------|-------------------------|------------------|
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